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Date: 10/12/01

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animals are also useful in further delineating useful treatment protocols for the conditions set out above.

Transgene construction. Polymerase chain reaction (PCR) primers were synthesized and used to amplify the fused FLINT-FLAG DNA sequences from plasmid pIG1-FLINTF, described above. The 5' primer, 5'-GAAGATCTTCTTTGATC AAGGATGGGCTTCTGGACTT-3', a BglII restriction site and the 3' primer, 5'-GGACTAGTCCTGATCATCACTTGTCGTCGTCGTCCTT-3', contained an SpeI restriction enzyme site. These were used to amplify the entire FLINT and FLAG coding region. The amplified 1.1 kb fragment was ligated into the multiple cloning site of plasmid pMTmcs2 generating plasmid pMTmcs2-FLINT (6.7 kb). See Fox et al, Eur. J. Pharmacol. 308:195 (1996).

The FLINT gene fragment was excised from pMTmcs2-FLINT by BglII and SpeI digestion and gel-purified. This fragment then was blunted with Klenow enzyme and ligated into the Klenow-blunted MluI site of plasmid pLIV.7, provided by John Taylor of the J. David Gladstone Institutes. See Fan et al., Proc. Nat'l Acad. Sci. 91:8724 (1994). Resultant plasmid pLIV7-FLINT also contains the apo E gene promoter/5' flanking region and an hepatic enhancer sequences called the "hepatic control region" (HCR). For microinjection into embryos, a 7.0 kb DNA fragment encompassing the Apo E gene promoter-FLINT/FLAG-HCR fusion gene was excised from plasmid pLIV7-FLINT by digestion with SalI and SpeI and purified by gel electrophoresis and glass bead extraction.

Transgenic animal development. Transgenic mice were generated using established techniques described, for example, by Hogan, B. et al. (1986) MANIPULATING THE MOUSE EMBRYO: A LABORATORY MANUAL, Cold Spring Harbor Laboratory (Cold Spring Harbor, NY), as modified by Fox and Solter, Molec. Cell. Biol. 8: 5470 (1988). Briefly, the 7.0 kb DNA fragment encompassing the Apo E gene promoter-FLINT-HCR fusion gene was microinjected into the male pronuclei of newly fertilized one-cell-stage embryos (zygotes) of the FVB/N strain. The